

# **Near IR Imaging on the UV510**

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### **Need to Bring Over To UV510**

All of these items are currently located in building 538/room 125. A slide box in the bottom bin of the refrigerator in the NLO510 room. The box is marked Near IR.

- 1. Small flat head screw driver with green handle.** This makes getting the C clamp off much easier.
- 2. Dish with Near IR beads.** Peak Flow - Flow Cytometry Reference Beads. Located in frig in NLO510 room. Bottom bin large slide box.
- 3. Chamber slide with Alexa 750 dye in it.** Frig in NLO510 room Located bottom bin large slide box. The stock solution is in the freezer of NLO510 room.
- 4. Rubber bands.**

### **Before Getting Near IR Imaging Set Up**

**If the user has a live cell experiment make sure there is water in the incubator .**

- 1. Use Standard Slide #3 to get a focus for experiment samples.** This should help make things easier when putting on sample.
- 2. Set up DIC using Standard Slide #3.** For experiment samples the 561 laser has been shown to give the best DIC.
- 3. Turn lamp off.** You may need to wait and let lamp housing cool before you can safely touch it to do the next part.

**There is no need to exit the software to change over to near IR.**

### **Switching to Near IR Set Up**

Will need two screw drivers from the tool kit located on the shelf in the LCI510 room:

1. Black handle SW 2,5 x100
2. Red handle SW 3,0 x100

You will also need gloves.

## **On Microscope**

If you wish to reuse settings use one of the Near IR folders in Kim's E:/CLSM/Kim folder. Most recent experiments are best.

**1. Make sure Cy7 filter cube is in place.** There is a filter cube box under the computer monitors. If the Cy7 filter is in it you will see Cy7 written on the cube. If not it's the triple band pass filter. **\*\*Careful handling the filter cubes. Use gloves and avoid touching the filter discs!!\*\*** If you need to change the filter cube you must remove the filter wheel from under the microscope turret. Again use care in handling the filter cubes.

**2. Remove the Mercury Lamp Housing from behind the microscope.**

**3. Remove safety lock mechanism from behind the microscope where the mercury lamp housing had been attached.** Tape this together so the pins are securely attached. Place a rubber band around the housing and safety lock. This is to make sure the laser safety lock is not triggered when using the microscope.



**4. Remove the Halogen Lamp from its normal place atop the microscope.** Make sure clamp is in place and pins are contacting. See page 7 for a clearer description of the safety lock mechanism in this area.

**5. Remove the heat filter from the Halogen Lamp.** First remove C Clip. Be careful that you do not touch or scratch the filter when you do this. Make sure you are wearing gloves and use Kimwipes when handling the heat filter so you do not get finger prints on the filter. Place filter in safe location. Small green handled flat head screw driver from 538/125 makes removing the C Clip easier.

**6. Attach the Halogen Lamp housing to the back of microscope.** Where the mercury lamp housing had been attached

**7. Make sure slider underneath the turret is moved so the polarizer is not blocking the light.**



## Imaging Samples:

To test the set up is working use a dish of Peak Flow - Flow Cytometry Reference Beads. Lid must be off sample to get DIC image.

## On Computer:

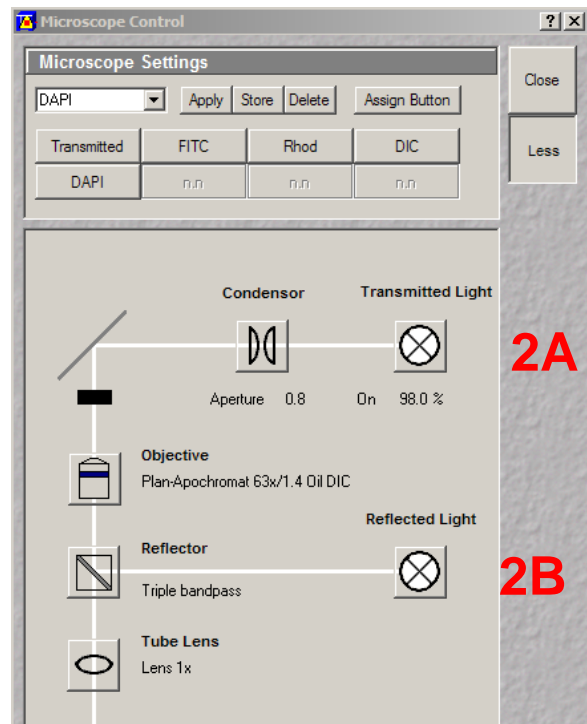
1. Make sure you are working in VIS



2. Microscope Control Window:

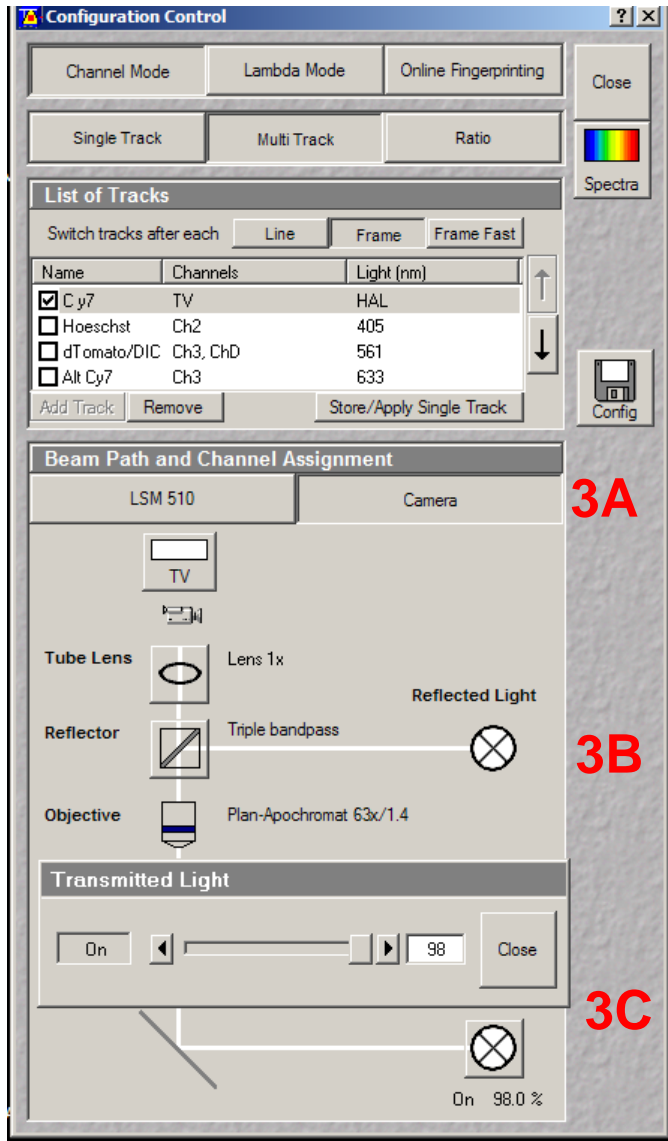
A) Transmitted on [look for light in housing to make sure it is working].

B) Turn on Reflected light and look for light coming up through the objective. **Make sure you have the Triple Band Pass Filter selected as this should have the Cy7 filter cube installed in its place.**



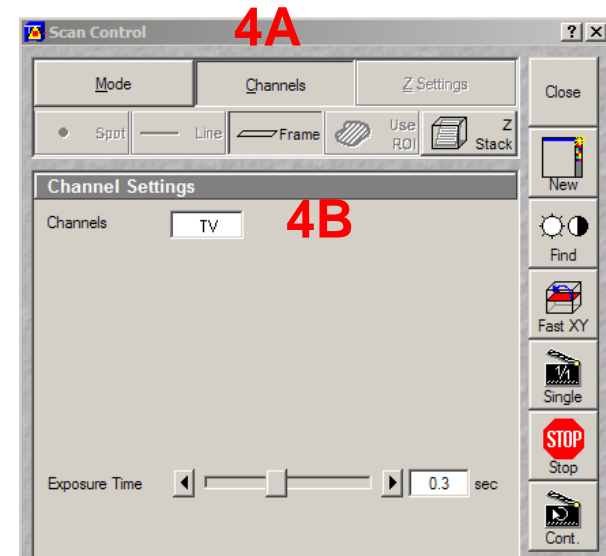
### 3. In Configuration Control Window:

- A. Set to Camera
- B. Make sure you are in Triple bandpass filter
- C. Click on Transmitted Light button and set slider to the right.



### 4. In Scan Control Window:

- A. Set to Channels
- B. Channels should be in TV
- C. Set exposure time



- 5. Click Continuous to get focus.** Remember this is different from regular imaging. You will need to be patient with focusing. It could take quite a bit of turning the focus knob to get in focus. Wait until image actually appears before moving focus again. The longer the exposure time the longer it will be between images.

**Spots on the image are on the microscope camera.**

**If this does not work check to see if laser is firing.** If laser is not firing make sure pins are in place on the laser safety locks. First check the pins are secure from the laser safety lock on the halogen lamp housing shown in “On Microscope Step 3”. Two other locations of laser locks are illustrated below.

When removing the halogen lamp check to see that the collar is in place tightly. The pins on the side should not be visible. If you get a laser safety lock warning this is one area to check



The laser lock for the microscope stand is located at the arrow in the image below. Make sure no cable is caught when the stand is lowered.



## DIC Image

**DIC image is best to find cells and get focus**

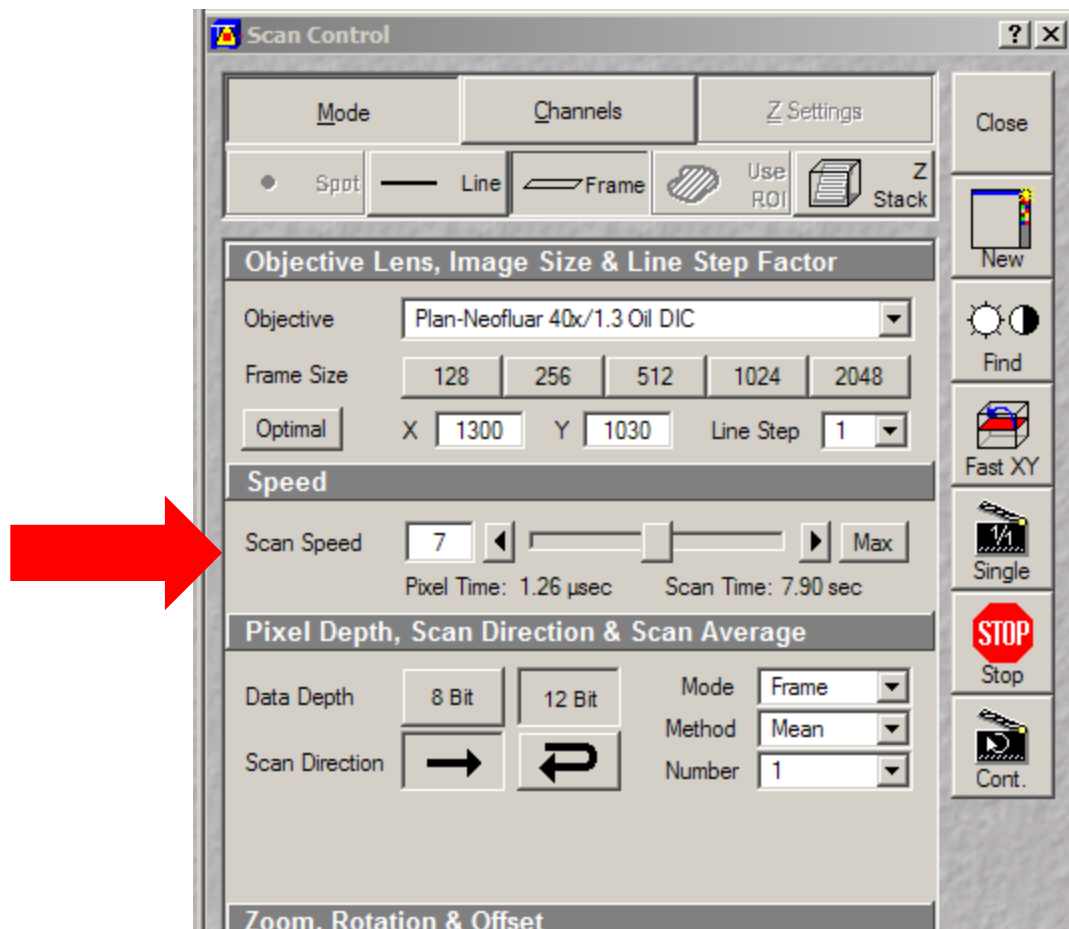
- 1. Your image will not be in the same orientation to confocal image so will not overlay unless you do some post-processing.** The confocal image is upside down relative to the camera image. You must also make sure to set the confocal and camera images to the same size or you will not be able to overlay them in post-processing. If you change the rotation of the image make sure you change it back.
- 2. You can remove camera noise from the images using the median filter.**
- 3. DIC Imaging works well with this method.**

**At any time if your image doesn't look right check the settings Be very vigilant about settings. They seem to switch without you knowing it. Especially make sure the triple band pass filter remains selected.**

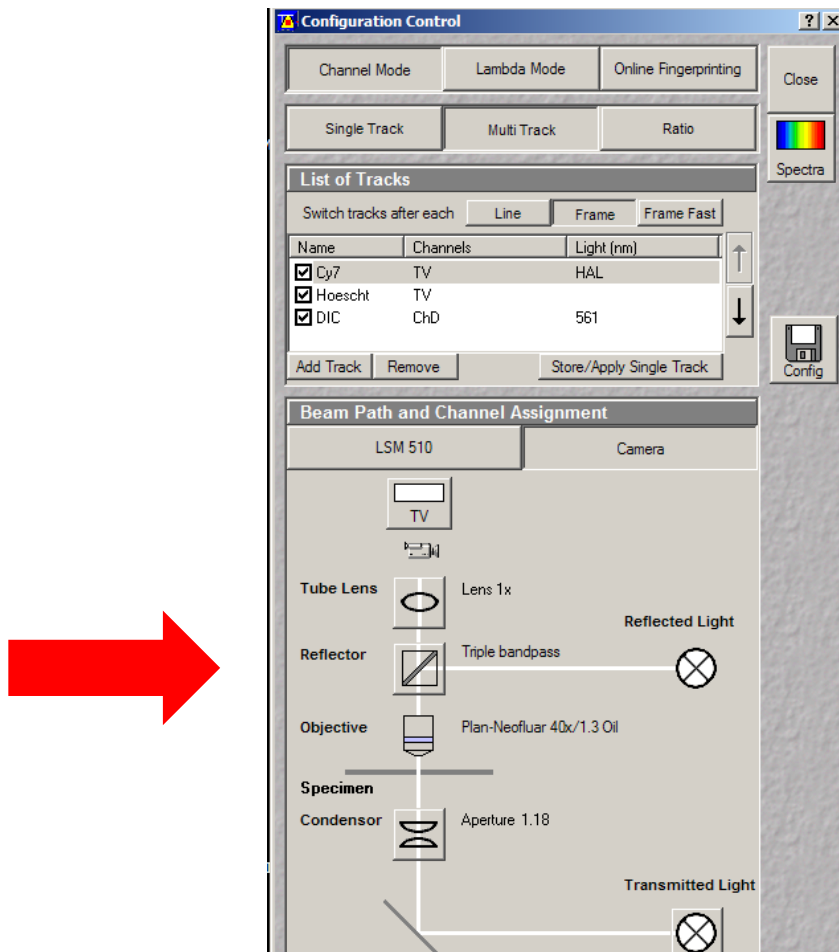
**If the fluorescent images are dimmer in single scan than in fast scan check the scan speed. Scan speed should be 7. See next page for screen shot.**



If the fluorescent images are dimmer in single scan than in fast scan check the scan speed. Scan speed should be 7.



**Light path set up for Cy7. Remember to make sure the Triple Bandpass filter is selected. This will indicate the position of the Cy7 filter cube.**



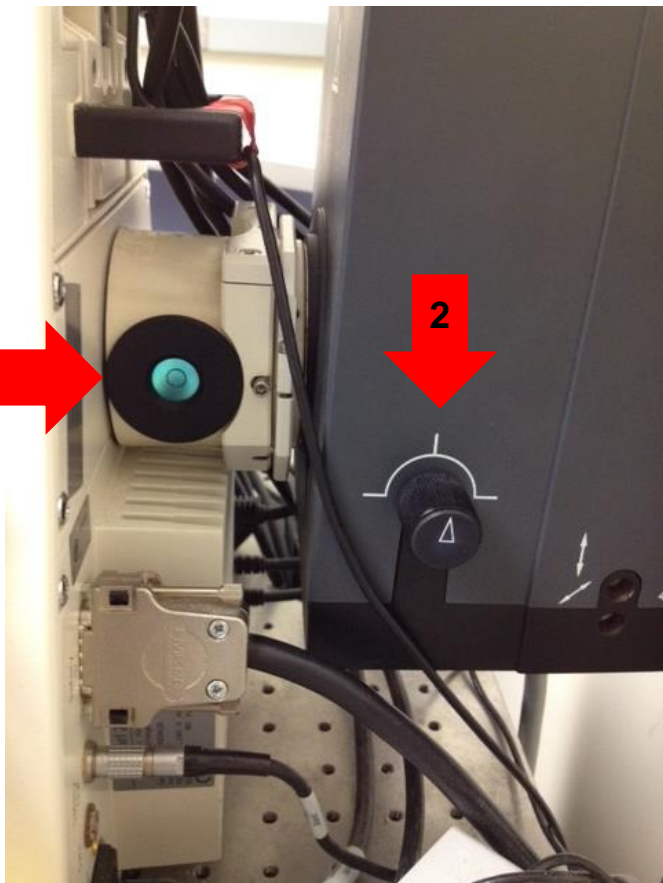
## **When Finished**

- 1. Return everything back to the way it was.** Refer to earlier pages for what needs changing back.
- 2. Be careful that Mercury Lamp housing is put on straight.**
- 3. Put standard slide on microscope to make sure you can see through the eyepiece and that the illumination is even.** If you do not see even illumination troubleshooting advice is on the next page.
- 4. Fast scan sample to make sure it is working ok.**

When checking through the eyepiece for even illumination keep these three places in mind if illumination is uneven.

1. The periscope is fully pushed in
2. The arrow on the knob is pointing up
3. The slider on the side of the microscope

If these three things are in the correct position you will have to use the screw driver to adjust the bulb position [#4 in image]



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# **ANALYSIS**

## Get MIPAV from <http://mipav.cit.nih.gov>

1. File -> open image from disk
2. Select files of type .ism at the bottom of the window.
3. Magnify image if too small: Image -> zoom -> 2X, then click on image
4. Separate stack of images into individual images: Utilities -> 3D slice tools -> extract slices. Click "select all", click "extract".
5. Enhance visual display of images. This does not affect the underlying data in the image: Click on image, LUT -> Histogram LUT. Increase the brightness of the image by moving up the small squares along the yellow line in the graph at the bottom of the window. Repeat for other images as necessary
6. Reflect the IR image. Click on the IR image. Utilities -> flip image -> horizontal. Click "horizontal Y axis" in the second row. Click OK.
7. Estimate shift between IR image and other images. Left click in the IR image to get the x and y coordinates. Repeat for the equivalent position in another image.
8. Edit scaling of IR image. Click on IR image. Image -> attributes -> edit attributes. Click on "resolutions". Set 1<sup>st</sup> dimension and 2<sup>nd</sup> dimension to 1. Press Apply.
9. Register IR image to other images by shifting the IR image. Click on IR image. Algorithm -> transformation tools -> transform. Check "user defined transformation matrix". Enter the amounts to shift in the x and y directions at Tx and Ty. May be a negative number. Press OK.
10. Check the registration. Utilities -> conversion tools -> grays -> rgb. Selection the shifted IR image as "red" and another image as "green". Set "blue" to "blank". Press OK. Will need to enhance visual display to see result: refer to (5) above.

11. Enhance DIC image. First change type of bytes to “double”. Click on DIC image. Utilities -> conversion tools -> convert type. Click “double” under image type. Press OK. Second smooth the new image. Click on the new image. Algorithms -> filters (spatial) -> Gaussian blur. Set x dimension and y dimension to 10.0 Press OK. Third, divide first image by the smoothed image. Click on the image from the first step. Utilities -> image calculator. Select “divide” for the operator. Choose the blurred image from step two for image B. Press OK.

12. Remove horizontal line artefacts from the image from step 11. First zoom and use enhance visual display (step (5)) to see if lines are present. If so, click on this image. Algorithms -> FFT. Very significantly enhance the FFT image and may need to change the color look up table (LUT), by clicking on a look up table in the second row. The one most on the right works best. Look for a vertical streak. Draw around the top part of the vertical streak using the VOI tools. Do not draw near the center of the image. Only draw in the top half. VOI -> New VOI -> draw rectangle VOI. Highlight VOI by clicking on it. Right click on “Fill VOI mask operation”, which is the third icon from the right on the second row. Set to “perform mask in place”. Then press left mouse button on the same icon. Next flip the VOI you just drew. If necessary, click on FFT image and click on VOI if not highlighted. VOI -> flip VOI -> VOIflipX. Highlight VOI by clicking on it. Then click on “Fill VOI mask operation” with the left mouse button. Almost there. Do inverse FFT: Algorithms -> FFT. Finally copy inverse FFT image to the correct size. Click on the image from step 11 and go to: Image -> attributes -> view header. Note the values for dimension 0 and dimension 1. I got 1300 and 1030. Do the same for the Inverse FFT image. I got 2048 and 2048.